Peripheral signals of food intake in response to low leptin levels induced by centrifugation

[1]M.M. Moran,[2] T.P. Stein, and [1]C.E. Wade

[1]Life Sciences Research Division, NASA Ames Research Center, MS 239-11, Moffett Field, CA, 94035, [2] Department of Surgery, University of Medicine and Dentistry of New Jersey, School of Osteopathic Medicine, Stratford, New Jersey, 08084

Running Head: Modulation of food intake in hypergravity

Corresponding author: Charles E. Wade

NASA Ames Research Center

MS 239-11

Moffett Field, CA 94035

Tel: (650) 604-3943

Fax: (650) 604-3954

Abstract

The focus of the study was to examine leptin and other peripheral signals of energy balance, following hypergravity. The study was conducted in two experiments. In experiment 1 rats were centrifuged at either 1.5, 2, or remained at 1 G. During days 8 to 14 of experiment 1, mean body mass of the 1.5 and 2 G groups was significantly ($p<0.05$) lower than controls. No differences were found in food intake (g/day/100 g body mass). Epididymal fat in the 2 G group was 21% lower than controls and 14% lower than the 1.5 G group. Plasma leptin was reduced from controls in the 1.5 and 2 G groups by 45 and 63% respectively. A significant correlation was found between G load and urinary catecholamines. In experiment 2, rats were centrifuged at either 1.25, 1.5, or remained at 1 G. During days 8 to 14, body mass and food intake were similar between the 1, 1.25, and 1.5 G groups. Epididymal fat was reduced from controls in the 1.25 (14%) and 1.5 (19%) G groups. Leptin was reduced from controls in the 1.25 (45%) and 1.5 (46%) G groups. No differences were found in urinary epinephrine. Urinary norepinephrine levels were significantly higher than controls in each centrifuge group. During hypergravity exposure, food intake is the result of a complex relationship between multiple pathways, which abates the importance of leptin as a primary signal.

Keywords: norepinephrine, epinephrine, body mass regulation, epididymal fat

INTRODUCTION

Modulation of appetite is a complex process. Several peripheral hormonal signals are integrated within the arcuate and paraventricular nuclei of the hypothalamus. These hormones control the production and release of specific neuropeptides, including neuropeptide Y (NPY), agouti related protein (AgRP), and alpha melanocyte-stimulating hormone (alpha-MSH) (7, 16, 19, 22). Recently, the *ob* gene was cloned from adipose tissue and its end-product leptin was implicated as a peripheral modulator of long-term energy balance (28).

Under normal and non-pathological conditions, the amount of leptin in the blood is a function of fat mass (2, 4, 10, 13, 18) and can therefore be implicated as a monitor of fat reserves. When leptin levels are elevated, NPY and AgRP production are inhibited, and at the same time alpha-MSH production increases, causing satiety (10, 23). When leptin levels are low NPY and AgRP increase, which inhibits alpha-MSH from binding and food intake increases (5). Thus when the satiety signal is not sensed by the hypothalamus by either low leptin levels or an inability to receive leptin's signal hyperphagia and obesity ensue (28). Although, leptin acts to monitor the status of our energy reserves, the exact nature and magnitude of its importance in regulating body weight remains unclear.

Centrifugation is a unique tool for studying body weight. During centrifugation, body weight is a function of the mass of the subject and the magnitude of the horizontal force vector that is produced by the speed of rotation and the distance of the subject from the axis of rotation. Thus, body weight can be increased immediately without manipulating food intake and/or activity. In a 1G

(terrestrial) environment, increases in food intake that exceed the amount of energy that is lost through exercise and/or activity cause body weight and fat mass to increase. However, despite the increase in body weight that is induced by centrifugation, food intake and fat mass decrease (9, 18, 19). The loss of fat should be paralleled by a similar reduction in plasma leptin. The purpose of the present study was to examine the effects of low leptin levels induced by centrifugation on other peripheral signals of food intake including, glucose, insulin, corticosterone, epinephrine, and norepinephrine.

METHODS

Before initiation of these studies, approval was received from the Institutional Animal Care and Use Committee (IACUC) at the National Aeronautics and Space Administration (NASA) Ames Research Center. The study conforms to NASA's *Animal Users Guide* and the National Research Council guidelines for animal experimentation. The centrifuge can accommodate two different G-loads at a time, thus the study was conducted in two experiments (1 & 2),

Study Design (Experiment 1)

The experiment was conducted using 1.5 month-old, male Sprague-Dawley derived albino rats (Simonsen Laboratories, Gilroy, CA). Upon receipt from the vendor, each rat was weighed and housed (1 rat/cage) in standard vivarium cages for a three day acclimation period. The acclimation period was followed by surgery, a seven day surgical recovery period, a seven day baseline data collection period, and a 14-day test period of either centrifugation at 2.0 G (12 ft. 21.1 RPM), 1.5 G (8ft, 21.1 RPM), or were stationary 1 G controls (n=8/group). Surgery consisted of implanting telemeters into each rats' abdomen (data not shown). Throughout the study the rats were maintained on a12:12-hour light dark cycle (06:00 on:18:00 off). Room temperature was maintained at 23 ± 2°C. Animals were fed a powdered diet (Purina Rat Chow no. 5012). Food and water were provided *ad libitum*. Daily data collection and animal health checks occurred at 08:00 and lasted for 45 minutes.

During the baseline and test periods, rats were housed (1 rat/cage) in metabolic cages (dimensions: length-width-height, 23" - 14" - 13'). Food and water were provided on the side of the cage to prevent contamination in the urine and feces. Water bottle lix-its were modified to prevent dripping during the starting and stopping of the centrifuge. Control rats were housed in the same room as the centrifuge rats.

Study Design (Experiment 2)

The design was similar to experiment 1 with the exception of the centrifuge groups. Rats were centrifuged at 1.5 G, (12 ft. 16.06 RPM), 1.25 G (8 ft, 16.06 RPM), or were stationary 1 G controls (n=8 rats/group). To account for physiological changes that may be due to rotation, the 1.5 G groups from experiments 1 and 2 were centrifuged at the same G load, however the radius and rate of rotation were different.

Urine Collection. Daily urine samples were collected from each rat. In each cage, urine was passed through a funnel, filtered by a urine and fecal separator, and collected into 30-ml conical tubes. To minimize evaporation, 1 ml of decahydronapthylene oil (Fisher Scientific, Pittsburgh PA) was added to each tube. At the end of the 24-hr collection period the tubes were brought to the lab, the samples were weighed, the oil was removed, and the samples were centrifuged. The samples were frozen at –20 °C. Urine catecholamine analysis was performed on pooled samples which were collected from each rat on days 11 to 14 of

experiment 1 and 2. Samples analysis was performed by high pressure liquid chromatography (HPLC) (DIONEX, Santa Clara CA). Catecholamine excretion rates were the product of the concentration multiplied by the mean volume excreted during a 24-hr period.

Dissection. A dissection was performed on each rat at day 14 of the test period. The rats were anesthetized with isoflurane and killed by decapitation. Prior to decapitation, blood was collected by cardiac puncture, kept on ice, centrifuged, and frozen for further analysis. Bilateral epididymal fat pads were collected, and weighed. Previous data collected in our laboratory has shown a high correlation between epididymal fat pad weight and total body fat in rats (r^2 =0.797). In addition, this technique has been proven a successful indicator of percent body fat in other rodents (4, 6).

Plasma Hormones. Commercial radioimmunoassay (RIA) kits were used to measure plasma leptin (ALPCO, Windham NH), insulin (Diagnostic Products Inc., Los Angeles CA), glucagon (Diagnostic Products Inc., Los Angeles CA), and corticosterone (ICN Biomedicals, Costa Mesa CA). For leptin, intra-assay variability was less than 5% and inter-assay variability was less than 8 %. The sensitivity of the plasma leptin assay was 0.6 pg/ml. The sensitivity of the insulin assay was 1.3 uIU/ml and both the intra- and inter-assay variability were less than 10 %. Corticosterone was measured with a double antibody RIA. The sensitivity

of the corticosterone assay was 12.5 pg/ml at the lowest standard level, intra-assay variability was 7.1 %, and the inter-assay variability was 6.5 %.

Statistics. All statistics were performed by using the Statistica software program (Statsoft, version 4.1, Tulsa OK). Data were compared by analysis of variance (ANOVA). If a significant difference ($P \leq 0.05$) was found by ANOVA, a Newman-Keuls post hoc test was performed. Regression analysis was used to probe for a dose response relationship between G load and the following parameters; 1) urinary catecholamines, 2) plasma leptin, 3) fat mass, 4) body mass. Each parameter was compared as a percentage of the controls. To account for differences due to rotation, data from 1.5 G groups were compared by ANOVA.

RESULTS

Food Consumption. In experiment 1, baseline food consumption was not different between the 1.0, 1.5, and 2.0 G groups (Table 1). During the first seven days of the centrifugation period, both the 1.5 and 2.0 G groups ate significantly less than controls. These changes were transient. There were no significant differences between groups on days eight through fourteen.

During experiment 2, food consumption followed a pattern similar to experiment 1 (Table 1). Baseline food consumption was similar between the controls, 1.25 and 1.5 G groups. During the first week of the test period food consumption was lower in the 1.25 and 1.5 G groups than the controls. These changes were transient. After eight days, food intake returned to control values.

Body Mass. During the baseline period of experiment 1, there were no differences in body mass between the 1.0, 1.5, and 2.0 G groups. Within the first seven days of centrifugation, body mass was reduced significantly in both the 1.5 and 2.0 G groups from controls. From day eight to day fourteen, the body mass of the centrifuged groups remained lower than controls. However, the rate of growth was similar at 4 ±1.2, 5 ±0.6, and 4 ±0.7 g/day in the 1.0, 1.5, and 2.0 G groups respectively.

During the baseline period of experiment 2, body mass was also similar between each of the three groups. However, body mass was significantly lower in the 1.25 and 1.5 G groups for the first week of the test period, and was not significantly different from controls after eight days.

Epididymal Fat Pad and Plasma Leptin. In experiment 1, there were no significant differences in fat pad weights between the 1.0 and 1.5 G groups (Fig. 1A). Centrifugation at 2.0 G however, resulted in a 21 % reduction in fat pad weight. Fat pad weight was 14 % less in the 2.0 than the 1.5 G group. Plasma leptin levels were 45 and 63 % lower in the 1.5 and 2.0 G groups respectively, than the controls (Fig. 1C). The 1.5 and 2.0 G groups had similar plasma leptin levels (Fig. 1C). In addition significant correlations were observed between epididymal fat mass and plasma leptin (r^2 =0.8768).

In experiment 2, the epididymal fat pad weights of 1.25 and 1.5 G groups were reduced by 14 and 19 % respectively (Fig. 1B). There were no differences between the 1.25 and 1.5 G groups (Fig. 1B). Plasma leptin followed a similar pattern. Centrifugation at 1.25 and 1.5 G reduced plasma leptin levels by 46 and by 45 % respectively (Fig. 1D). No significant differences in leptin levels were observed between the centrifuge groups (Fig. 1D). In addition significant correlations were observed between epididymal fat mass and plasma leptin (r^2 =0.8245).

Plasma Analysis. No differences were found between any of the groups in plasma glucose, insulin, or corticosterone in either experiment (Table 2).

Urinary Catecholamines. Experiment 1. Urinary norepinephrine and epinephrine were significantly higher in both the 1.5 and 2.0 G groups than the 1.0 G controls (Fig. 2A). In addition, urinary norepinephrine and epinephrine were significantly higher in the 2.0 G compared with the 1.5 G group (Fig. 2A).

Experiment 2. There were no differences in urinary epinephrine between the 1.0, 1.25, and 1.5 G groups (Fig. 2B). There were no differences in urinary norepinephrine levels between the 1.25 G group and controls (Fig. 2B). The 1.25 G and 1.5 G groups exhibited similar urinary norepinephrine levels, however there was a significant difference between the 1.5 G and control groups (Fig. 2B).

Dose and Rotational Responses

Dose Response: When the data from each experiment were compared as a percentage of 1.0 G controls, significant dose-response relationships were found between G load and the following parameters; body mass, fat mass, plasma leptin, urinary epinephrine, and urinary norepinephrine (Fig. 3).

Rotational Effects: To evaluate the rotational effects of centrifugation, the two 1.5 G groups were compared. The difference between the groups was in the speed of rotation and distance of the rats from the axis of rotation. In experiment 1, the 1.5 G group was centrifuged at a rate of 21.1 RPM, and was housed 8 ft from the axis.

In experiment 2, the 1.5 G group was centrifuged at a rate of 16.06 RPM, and was housed 12 ft from the axis. No differences were found in the following parameters; body mass, food consumption, body fat, plasma insulin, or plasma corticosterone. Differences were found in plasma leptin and glucose, urinary epinephrine and norepinephrine. It is likely that these were population differences, as the change from 1.0 G controls was similar and both 1.5 G groups (Fig. 3).

DISCUSSION

Increases in body weight due to centrifugation result in an initial loss of body mass that is dependent upon gravity level, and primarily due to a reduction in fat mass. Following 14-days of centrifugation, the loss of fat was paralleled by reduction in plasma leptin, and would be expected to promote hyperphagia. However, no differences were observed in food intake. The absence of hyperphagia in the presence of low plasma leptin was puzzling, in light of leptin's inhibitory effect on food intake. The primary focus of the study was to examine selected peripheral food intake modulators in the presence of low leptin levels.

The decrease in food consumption and body mass within the first seven days of centrifugation were expected (18, 21, 26, 27). These changes were transient, and centrifuged rats subsequently gained mass at a rate similar to controls. Although there were no differences in body mass between the groups from experiment 2, differences were found in experiment 1 thus, food intake data was normalized to each animal's body mass.

The loss of body fat caused proportional reductions in plasma leptin, as a direct correlation between these two parameters was demonstrated. It is likely that the lypolysis was triggered by a rise in circulating catecholamines at the onset of centrifugation. Although these data were not presented, the onset of centrifugation invokes a stress response and would therefore result in an elevation in circulating catecholamines (17). The most interesting result was the disparity between food intake and the plasma leptin levels, as centrifuged animals ate an amount that was

similar to controls (Table 1). Thus, another peripheral modulator of food intake must act on the system to prevent hyperphagia.

Leptin and insulin are believed to act in a similar manner to inhibit food intake (14). The reduction in plasma leptin could be compensated by an increase in glucose tolerance. Previous data have shown that rats exposed to seven months of centrifugation at 4.15 G have an enhanced glucose tolerance (15), which may provide further support for hypophagia and prevention of obesity during centrifugation. We did not observe any differences between groups in plasma glucose or insulin, which indicated that insulin stimulated uptake of glucose remained intact at each G load. Furthermore the system was able to maintain energy intake at a level that supported growth.

Circulating glucocorticoids levels rise at meal times (3, 25) and act to stimulate food intake and leptin production. However, appetite inhibition has not been found with glucocorticoid induced rises in circulating leptin (8). Furthermore glucocorticoids prevent leptin induced inhibition of food intake and will reverse decreases in food intake that are normal responses to exogenous leptin administration (22). The consequences of interactions between low levels of leptin and glucorticoids in modulating food intake remain unclear. No differences in corticosterone were found between groups. Thus it is difficult to ascertain a role of corticosterone in this study.

Catecholamines play significant roles in regulating energy intake. Urinary norepinephrine levels in the 1.5 and 2.0 G groups were significantly higher than controls and similar norepinephrine levels were found between the 1.25 G and

control groups. The actions of norepinephrine are two-fold. At the systemic level, norepinephrine binds with adipocyte beta-3-AR and inhibits leptin gene expression, which causes a subsequent rise in food intake (12). It is unlikely that this pathway would affect food intake as fat mass decreased significantly. At the central level, norepinephrine reduces food intake (1). Similarly, epinephrine causes a reduction in food intake (11). Urinary epinephrine levels were elevated in the 1.5 and 2.0 G groups from experiment 1. Significant correlations were found between G load and urinary catecholamine levels (Fig. 3). It is possible that the increases in both norepinephrine and epinephrine prevented rats from overeating when plasma leptin was reduced and emphasize to the importance of catecholamines in achieving a new steady state during exposure to altered environments.

Despite the similarities in food intake between the control, 1.25, 1.5, and 2.0 G groups, there were a variety of physiological changes that responded in a dose response manner to increases in G-load (Fig. 3). Moreover, these data demonstrate that during exposure to changes in gravitational load, body mass is tightly regulated by a complex interaction between multiple peripheral signals. It is important to note that the reported data focus on peripheral effects. However it is the dichotomy of these afferent signals that is of interest. For example, we know that in the presence of low leptin levels, no significant changes in glucose, insulin, or corticosterone occurred. It is possible that catecholamines via the beta-3 AR may counterbalance these effects, and be implicated as important modulator of food intake.

The unique environment imposed by centrifugation is optimal for studying body weight regulation. While other studies have used manipulations in food intake and/or activity levels to examine body weight, this study manipulated body weight directly allowing examination of food intake and selected afferent signal pathways. Food intake was maintained despite the reduction in plasma leptin. It is likely that the binding of catecholamines to beta-3 AR acts as a primary signal to inhibit food intake. In addition, an enhanced glucose uptake may act in a similar manner to inhibit food intake. In conclusion, when body weight is increased by centrifugation, leptin is a minor component of the complex physiological circuitry, which regulates food intake.

Acknowledgments

We would like to thank Fang Yuan, Tom Wang, Lisa Baer, Heather Jonasson, Warren Belisle, and Mari Eyestone for assistance with data collection. We would also like to thank the Facilities Branch of the NASA Ames Life Sciences Division for centrifuge set-up and Dr. Carolyn Reed, David Garcia, and Mary Williams for Veterinary support. The results of this study have been presented at the 1999 International Space and Gravitational Physiology Annual Meeting.

Support was provided by NASA grants: 121-10-50, 121-10-30

Address for reprint requests: M.M. Moran, NASA Ames Research Center, Mail Stop 239-11, Moffett Field, CA., 94035-1000.

References

1. Bray GA, and York DA. The MONA LISA hypothesis in the time of leptin. *Recent Prog Horm Res* 53: 95-117, 1998.

2. Considine, RV, Sinha, MK, Heiman, ML, Kriauchunas, A, Stephens, TW, Nyce, MR, Ohannesian, J., Marco, CC, McKee, L.J, Bauer, TL, and Caro JF. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 334: 292-295, 1996.

3. Dallman, MF, Strack, AM, Akana, SF, Bradbury, MJ, Hanson, ES, Scribner, KA, and Smith M. *Frontiers in Neuroendocrinology*, New York: Raven, 1993, p 303-347.

4. Eisen, EJ, and Leatherwood JM. Predicting percent fat in mice. *Growth* 45(2): 100-107, 1981.

5. Fan, W, Boston, B, Kesterson, R, Hruby, V, and Cone R. Role of melancortinergic neurons in feeding and the agouti obesity syndrome. *Nature* 385: 165-168, 1997.

6. Friedman JM. The alphabet of weight control. *Nature* 385: 119-120, 1997.

7. Hamilton, BS, Paglia, D, Kwan, YM, and Deitel M. Increased obese mRNA expression in omental fat cells from massively obese humans. *Nature Med* 1: 953-956, 1995.

8. Jacobsen, L, Glucocorticoid replacement, but not CRH deficiency, prevents adrenalectomy-induced anorexia in mice. *Endocrinology* 140: 310-317, 1999.

9. Keil, LC. Changes in growth and body composition of mice exposed to chronic centrifugation. *Growth* 33: 83-88, 1969.

10. Klein, S, Coppack, SW, Mohamed-Ali, V, and Landt M. Adipose tissue leptin production and plasma leptin kinetics in humans. *Diabetes* 45: 984-987, 1996.

11. Leibowitz, SF. Brain monoamines and peptides: role in the control of eating behavior. *Fed Proc* 45(5): 1396-1403, 1986.

12. Li, H, Matheny, M, and Scarpace PJ. Beta 3-adrenergic-mediated suppression of leptin gene expression in rats. *Am J Physiol* 272(6 pt 1): E1031-E1036, 1997.

13. Maffei, M, Halaas, J, Ravussin, E, Pratley, RE, Lee, GH, Zhang, Y, Fei, H, Kim, S, Lallone, R, and Ranganatha S. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nat Med* 1(11): 1155-1161, 1995.

14. Mizuno, TM, Bergen, H, Funabashi, T, Kleopoulos, SP, Zhong, YG, Bauman, WA, and Mobbs CV. Obese gene expression: reduction by fasting and stimulation by insulin and glucose in lean mice, and persistent elevation in acquired (diet-induced) and genetic (yellow agouti) obesity. *Proc Nat Acad Sci USA*. 93: 3434-3438, 1996.

15. Mondon, CE, Dolkas, CB, and Oyama J. Enhanced skeletal muscle insulin sensitivity in year-old rats adapted hypergravity. *Am J Physiol* 240 3: E482-E488, 1981.

16. Mountjoy, K, Mortrud, M, Low, M, Simerly, R, and Cone R. Localization of the melanocortin-4 receptor (MC4-R) in neuroendocrine and autonomic control circuits in the brain. *Mol Endocrinol* 8: 1298-1308, 1994.

17. Ortiz, RM, Wang, TJ, and Wade CE. Influence of centrifugation and hindlimb suspension on testosterone and corticosterone excretion in rats. *Aviat Space Environ Med* 70(5): 499-504, 1999.

18. Oyama, J, and Platt WT. Effects of prolonged centrifugation on growth and organ development of rats. *Am J Physiol* 209: 611-615, 1965

19. Pitts, GC, Bull, LS, and Oyama J. Effect of chronic centrifugation on body composition in the rat. *Am J Physiol* 223:1044-1048, 1972.

20. Rossi, M, Kim, MS, Morgan, DG, Small, CJ, Edwards, CM, Sunter, D, Abusnana, S, Goldstone, AP, Russell, SH, Stanley, SA, Smith, DM, Yagaloff, K., Ghatei, MA, and Bloom SR. A C-terminal fragment of Agouti-related protein increases feeding and antagonizes the effect of alpha-melanocyte stimulating hormone in vivo. *Endocrinology* 139 (10): 4428-4431, 1998.

21. Smith, AH. Effects of chronic acceleration in animals. *Life Sci Space Res* 11: 201-206, 1973.

22. Solano, JM, and Jacobson L. Glucocorticoids reverse leptin effects on food intake and body fat in mice without increasing NPY mRNA. *Am J Physiol* 277 (Endocrinol. Metab. 40): E708-E716, 1999.

23. Stanley, BG, Kyrkouli, SE, Lampert, S, and Leibowitz SF. Neuropeptide Y chronically injected into the hypothalamus: a powerful neurochemical inducer of hyperphagia and obesity. *Peptides* 7: 1189-1192, 1986.

24. Stephens, TW, Basinski, M, Bristow, PK, Bue-Valleskey, JM, Burgett, SG, Craft, L., Hale, J, Hoffmann , J, Hsiung, HM, and Kriauciunas A. The role of

neuropeptide Y in the antiobesity action of the obese gene product. *Nature* 377(6549): 530-532, 1995.

25. Tempel, DL, and Leibowitz SF. Adrenal steroid receptors: interactions with brain neuropeptide systems in relation to nutrient intake and metabolism. *J Neuroendocrinol* 6: 479-501, 1994.

26. Thorling, EB, and Fredens K. The influence of small changes in the gravitational field on the weight regulation in female rats. *Inter Nat J Obes* 19: 305-309, 1995.

27. Warren, LE, Horwitz, BA, and Fuller CA. Gravity and body mass regulation. *J Grav Physiol* 4(2): 89-92, 1997.

28. Zhang, Y, Proenca, R, Maffei, M, Barone, M, Leopold, and Friedman JM. Positional cloning of the mouse *ob* gene and its human homologue. *Nature* 372: 425-432, 1994.

Figures:

Figure 1. A) Comparison of epididymal fat pad weight between 1 (open bars), 1.5 (gray bars), and 2.0 G (black bars) on day 14 of part 1. B) Comparison of epididymal fat pad weight between 1 (open bars), 1.25 (slashed bars), and 1.5 G (gray bars). C) Comparison of mean plasma leptin concentration between 1 (open bars), 1.5 (gray bars), and 2.0 G (black bars) on day 14 of part 1. D) Comparison of mean plasma leptin concentration between 1 (open bars), 1.25 (slashed bars), and 1.5 G (gray bars). Values are group means + SE. * Denotes a significant difference from 1 G controls. ** Denotes a significant difference from 1.5 G rats in experiment 1.

Figure 2. Comparison of 24-hour urinary epinephrine (open bars) and norepinephrine (closed bars) excretion from experiment 1 A) and experiment 2 B). Values are pooled samples collected from each rat on days 11-14 of both studies and are group means +se.

Figure 3. Regression analysis of urinary epinephrine A) urinary norepinephrine B) plasma leptin C) epididymal fat pad mass D) and body mass E) at 1, 1.25, 1.5, and 2.0 G. Values are group means ± se and were compared as a percentage of 1 G controls.

Table 1. Food Intake and Body mass during experiments 1 and 2.

Group	Food Consumption (g food /100 g body mass)			Body Mass (g)		
Experiment 1	Baseline	Early	Late	Baseline	Early	Late
1.0 G	10.0 ±0.2	8.9 ±0.1	8.0 ±0.1	246 ±2	280 ±3	314 ±4
1.5 G	9.6 ±0.1	7.1 ±0.1*	8.0 ±0.1	246 ±2	250 ±3*	283 ±3*
2.0 G	9.5±0.2	7.1 ±0.2*	7.7 ±0.2	247 ±2	246 ±3*	273 ±3*
Experiment 2						
1.0 G	9.8 ±0.2	8.6 ±0.1	7.4 ±0.1	238 ±3	268 ±6	301 ±10
1.25 G	9.5 ±0.1	6.9 ±0.1*	7.6 ±0.2	236 ±2	241 ±3*	279 ±3
1.5 G	9.4 ±0.1	6.9 ±0.1*	7.8 ±0.1	240 ±2	246 ±3*	283 ±4

Comparison of food intake and body mass during the baseline (three days prior to the start of the centrifuge), early (days one to seven of centrifugation), and late (days eight to fourteen of centrifugation) of experiments 1 and 2. Values are group means ± se. Comparisons were made within each experiment and * denotes a significant ($p \leq 0.05$) difference from 1 G controls.

Table 2. Experiment 1 and 2, plasma glucose, insulin, and corticosterone

Group	Glucose (mg/dl)	Insulin (ulU/ml)	Corticosterone (ng/ml)
Experiment 1			
1	159 ±7	6.8 ±.7	135 ±27.7
1.5	156 ±10	8.8 ±1.7	113 ±26.4
2	160 ±8	10.1 ±0.8	196 ±40.6
Experiment 2			
1	177 ±6	12.7 ±1.6	112 ±21
1.25	195 ±13	8.3 ±0.7	96 ±23
1.5	197 ±13	7.8 ±0.5	99 ±28

Comparison of plasma glucose, insulin, and corticosterone levels following 14 days of centrifugation to 1 G controls. Comparisons were made within each experiment and are group means ± se.

Figure 1

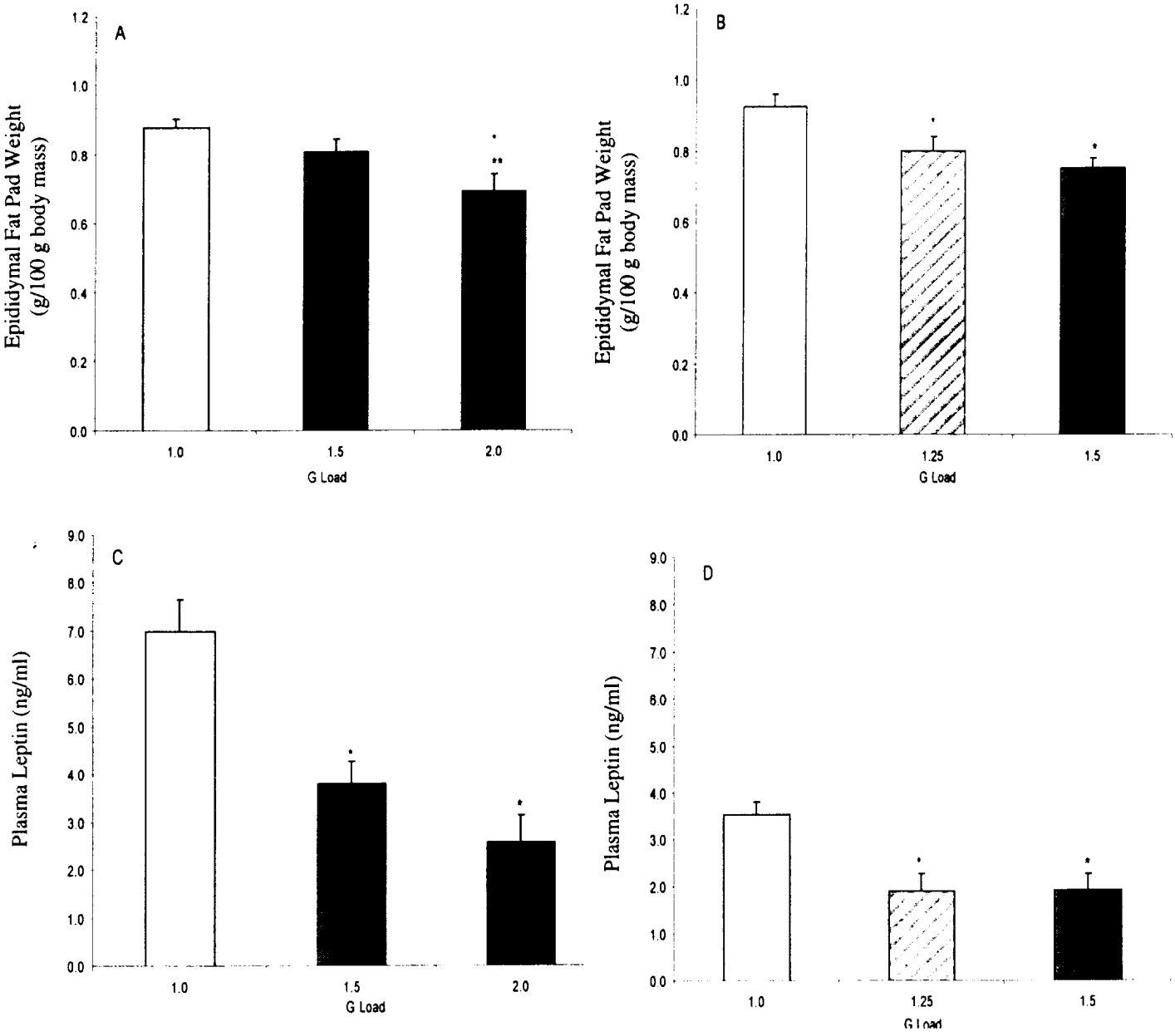

A
Epididymal Fat Pad Weight (g/100 g body mass)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1.0
1.5
2.0
G Load
B
Epididymal Fat Pad Weight (g/100 g body mass)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
1.0
1.25
1.5
G Load
C
Plasma Leptin (ng/ml)
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
1.0
1.5
2.0
G Load
D
Plasma Leptin (ng/ml)
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
1.0
1.25
1.5
G Load

Figure 2

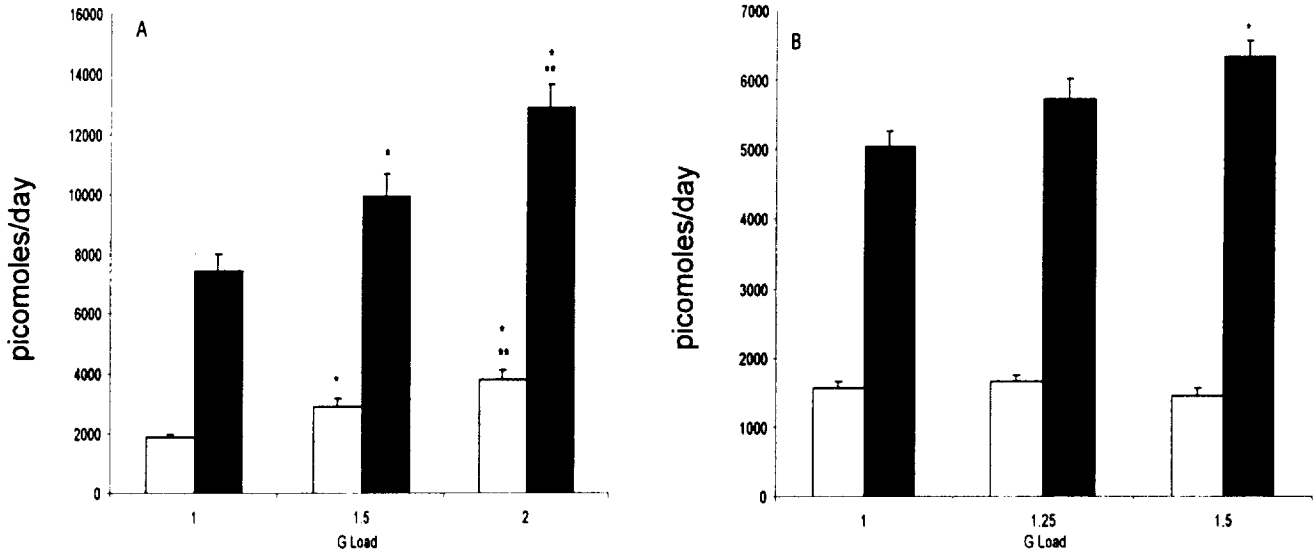
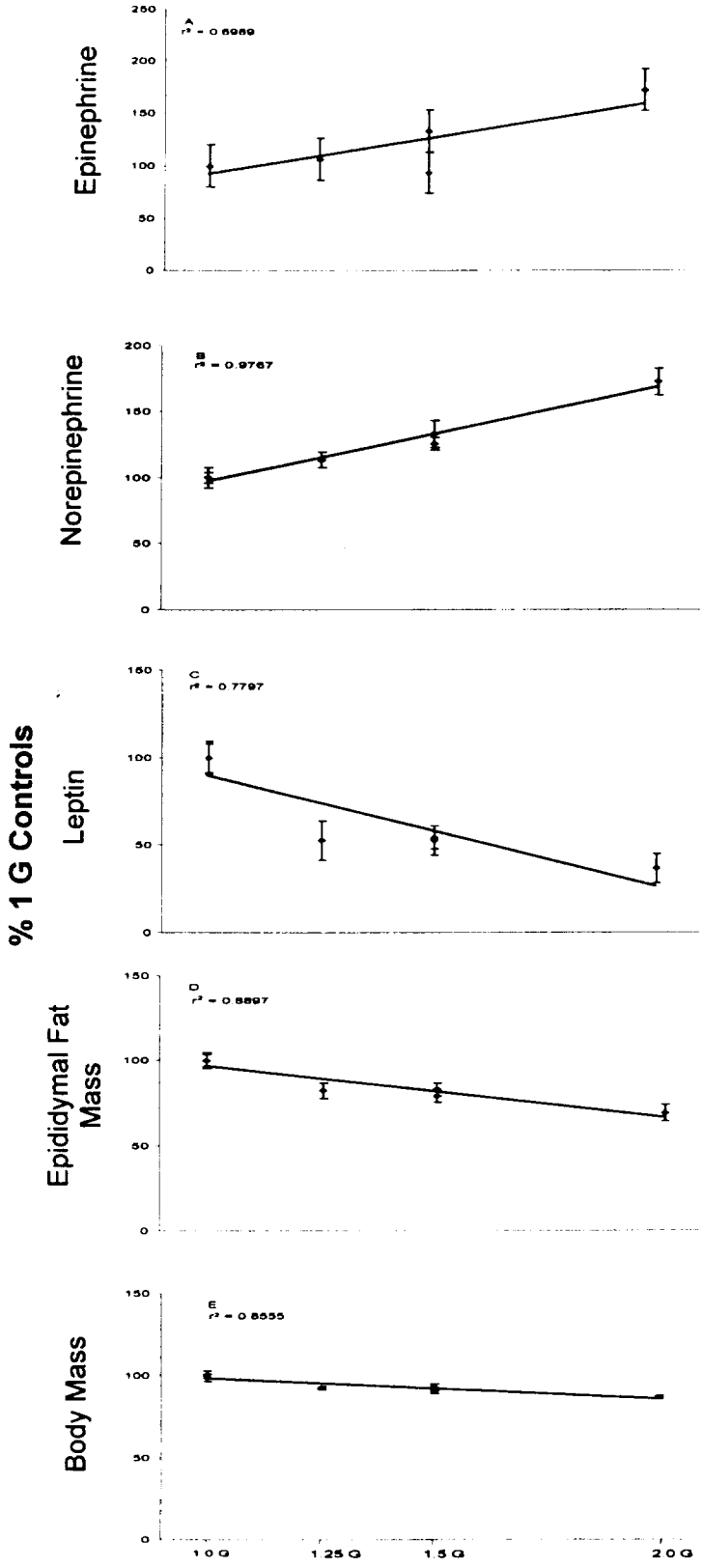

A
picomoles/day
16000
14000
12000
10000
8000
6000
4000
2000
0
1
1.5
2
G Load
B
picomoles/day
7000
6000
5000
4000
3000
2000
1000
0
1
1.25
1.5
G Load

Figure 3


% 1 G Controls
Epinephrine
A
r² = 0.6969
250
200
150
100
50
0
Norepinephrine
B
r² = 0.9767
200
150
100
50
0
Leptin
C
r² = 0.7797
150
100
50
0
Epididymal Fat Mass
D
r² = 0.8897
150
100
50
0
Body Mass
E
r² = 0.8555
150
100
50
0
1 0 G
1.25 G
1.5 G
2 0 G